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(54) Title: MAGE-10 ENCODING cDNA, THE TUMOR REJECTION ANTIGEN PRECURSOR MAGE-10, ANTIBODIES SPECIFIC TO THE MOLECULE, AND USES THEREOF			
(57) Abstract			
Isolated cDNA molecules which encode the tumor rejection antigen precursor MAGE-10, the protein itself, antibodies to it, and uses of these are a part of the invention.			

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**MAGE-10 ENCODING cDNA, THE TUMOR REJECTION ANTIGEN
PRECURSOR MAGE-10, ANTIBODIES SPECIFIC TO
THE MOLECULE, AND USES THEREOF**

FIELD OF THE INVENTION

5 This invention relates to tumor rejection antigen precursors, the nucleic acid molecules encoding them, antibodies specific to these, and uses thereof.

BACKGROUND AND PRIOR ART

10 The study of the recognition or lack of recognition of cancer cells by a host organism has proceeded in many different directions. Understanding of the field presumes some understanding of both basic immunology and oncology.

15 Early research on mouse tumors revealed that these displayed molecules which led to rejection of tumor cells when transplanted into syngeneic animals. These molecules are "recognized" by T-cells in the recipient animal, and provoke a cytolytic T-cell response with lysis of the transplanted cells. This evidence was first obtained with tumors induced *in vitro* by chemical carcinogens, such as methylcholanthrene. The antigens expressed by the tumors and which elicited the T-cell response were found to be different for each tumor. See Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970) for general teachings on inducing tumors with chemical carcinogens and differences in cell surface antigens. This class of antigens has come to be known as "tumor specific transplantation antigens" or "TSTAs".
20 Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar results were obtained when tumors were induced *in vitro* via ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

While T-cell mediated immune responses were observed for the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See 5 Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

The family of tum⁺ antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980), the disclosure of which is incorporated by reference. To elaborate, tum⁺ antigens are obtained by mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors (i.e., "tum⁺" cells). When these tum⁺ cells are 10 mutagenized, they are rejected by syngeneic mice, and fail to form tumors (thus "tum⁻"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977), the disclosure of which is incorporated by reference. Many tumor types have been 15 shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

It appears that tum⁺ variants fail to form progressive tumors because they initiate an immune rejection process. The evidence in favor of this hypothesis includes the ability of "tum⁻" variants of tumors, i.e., those which do 20 not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl. Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum⁺ cells of mastocytoma P815 multiply exponentially for 12-15 days, 25 and then are eliminated in only a few days in the midst of an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory which permits them to resist subsequent challenge to the same tum⁺ variant, even when immunosuppressive amounts of 30 radiation are administered with the following challenge of 35

cells (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra).

Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983). Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearon et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized by cytolytic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies, in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and the cells presenting the tumor rejection antigens are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the tumor rejection antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This type of analysis is required for other types of antigens recognized by CTLs, including minor histocompatibility antigens, the male

specific H-Y antigens, and the class of antigens referred to as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990), the disclosures of which are incorporated by reference. The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum⁻ variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille, supra). In contrast to tumor rejection antigens - and this is a key distinction - the tum⁻ antigens are only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be tum⁺, such as the line referred to as "P1", and can be provoked to produce tum⁻ variants. Since the tum⁻ phenotype differs from that of the parent cell line, one expects a difference in the DNA of tum⁻ cell lines as compared to their tum⁺ parental lines, and this difference can be exploited to locate the gene of interest in tum⁻ cells. As a result, it was found that genes of tum⁻ variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303 (1989). This has proved not to be the case with the TRAs of this invention. These papers also demonstrated that peptides derived from the tum⁻ antigen are presented by the L^d molecule for recognition by CTLs. P91A is presented by L^d, P35 by D^d and P198 by K^d.

PCT application PCT/US92/04354, filed on May 22, 1992 assigned to the same assignee as the subject application, teaches a family of human tumor rejection antigen precursor

coding genes, referred to as the MAGE family. Several of these genes are also discussed in van der Bruggen et al., Science 254: 1643 (1991). It is now clear that the various genes of the MAGE family are expressed in tumor cells, and can serve as markers for the diagnosis of such tumors, as well as for other purposes discussed therein. See also Traversari et al., Immunogenetics 35: 145 (1992); van der Bruggen et al., Science 254: 1643 (1991) and De Plaen, et al., Immunogenetics 40: 360 (1994).

10 U.S. Patent No. 5,342,774, cited supra and incorporated by reference, teaches various members of the MAGE family of TRAPs, in genomic DNA and cDNA form. Genomic DNA for MAGE-10 is taught in PCT application PCT/US92/04354, cited supra, in SEQ ID NO: 22, as a 920 base pair fragment. DePlaen, et al., Immunogenetics 40: 360-369 (1994), discusses PCR work which identified a 485 nucleotide portion of MAGE-10. Also, see Genbank Accession No. U10685, incorporated by reference. A cDNA molecule, however, is not discussed.

20 The previously cited PCT application discusses antibodies to MAGE proteins generally. Chen et al., U.S. Patent No. 5,541,104, to Chen et al., incorporated by reference, teaches monoclonal antibodies which specifically bind to tumor rejection antigen precursor MAGE-1. This patent is incorporated by reference. In order to prepare the monoclonal antibodies, Chen et al produced a MAGE-1 TRAP in E. coli which was not full length, because of difficulties with expression of the full length molecule.

25 It has now been found, however, that monoclonal antibodies which bind to both MAGE-1 and MAGE-10 TRAP can be produced. This is surprising in view of the reports in the literature, because it was not seen to be possible to produce such antibodies with the available information on MAGE-10. The TRAP encoded by the cDNA for MAGE-10 is found to be a molecule of about 72 kilodaltons molecular weight, on SDS-PAGE. It has also been found that polyclonal antibodies specific to MAGE-10 can be produced. These, as

well as other aspects of the invention, are set forth in the disclosure which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents results of a Western blotting assay,
5 using electrochemiluminescence detection to test reactivity
of monoclonal antibodies with various cell lysates.

Figure 2 presents results of tests designed to determine if
cell line NA8-MEL could be induced to produce 72 kilodalton
protein in the presence of MAGE-1 cDNA.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

Full length recombinant MAGE-1 protein was prepared in the form of a fusion protein, in *E. coli*. See Schultz-Thater, et al., Int. J. Cancer 59: 435-439 (1994), incorporated by reference. Briefly, full length MAGE-1 cDNA was cloned into a well known expression vector, pET 16b. This vector permits expression of a fusion protein which contains 10 histidine molecules at the N-terminus. The *E. coli* were cultured, following Schultz-Thater, after which the cells were lysed, and the recombinant fusion protein was purified on a Ni²⁺ column. The purified material, when tested by SDS-PAGE, showed a major band of 48 kilodaltons. This 48 kD material was used in the experiments which follow.

Example 2

Following the production of the recombinant MAGE-1 fusion protein, a BALB/c mouse was immunized intraperitoneally, twice, with 20 ug of the recombinant protein each time, in a composition which contained complete Freund's adjuvant. This was followed by two additional injections, each of 20 ug of recombinant MAGE-1, with incomplete Freund's adjuvant. The spleen cells of the

mouse were then fused with NS-1 myeloma cells, in accordance with Carrel, et al., Cancer Res. 40: 2523-2528 (1980). The resulting hybridoma cells were cultured, and supernatants from the cultures were screened, using an 5 ELISA, to determine if recombinant MAGE-1 specific monoclonal antibodies were being produced. The ELISA involved coating recombinant MAGE-1 protein (250 ng/50 ul per well), followed by overnight incubation at 4°C. Samples of supernatant were added, followed by biotin 10 conjugated sheep antimouse Ig, and streptavidin-alkaline phosphatase conjugate.

The ELISA resulted in the identification of 289 hybridomas which produced antibodies against recombinant MAGE-1.

15 Example 3

In the next set of experiments, the antibodies were tested to determine if they could be used to immunostain cells which were positive for mRNA for MAGE-1.

Initially the hybridomas were screened to try to eliminate any cross reactive monoclonals. To do this, cell 20 lines with known, and different patters of MAGE-TRAP expression were tested. MZ2-MEL 3.1 is known to express all of MAGE-1, 2, 3 and 4; MZ2-MEL 2.2 expresses MAGE-2 and 25 3; and U251, a glioblastoma cell line negative for all four, were tested. Cells were cultured in 16 well plastic chambers, fixed in cold acetone (0°C for five minutes), and then stored until ready to use at -20°C. Endogenous peroxidase was then blocked with 0.3% H₂O₂ (10 minutes), and the cells were then preincubated, in 0.1% bovine serum 30 albumin in phosphate buffered saline, for 30 minutes. This produced a first layer of a three layer biotin/avidin/peroxidase system as described by Carrel, et al., supra. Following the fixing of the cells, goat anti-mouse IgG biotin conjugate was added (following 1:50 dilution), to yield the second layer. Finally, avidin-peroxidase conjugates were added, following dilution at 35

1:1000. In the case of the second and third layers, incubation was for 30 minutes and then 15 minutes. Peroxidase was visualized with amino-ethylcarbazole, and counter staining of cells, using Gill's hematoxylin for 30 seconds. This set of experiments results in the discovery that two mAbs, i.e., 6C1 and 6F2, stained only the MZ2-MEL 3.1 cells. These two clones were then used in a series of experiments on cells which had been tested for mRNA for MAGE-1, 2, 3 and 4. Cells were classified as being positive or negative for MAGE-1 mRNA expression. This was determined by following the procedures of Rimoldi et al., Int. J. Cancer 54: 527-528 (1993); Brasseur et al., Int. J. Cancer 63: 375-380 (1995). In brief, total RNA was extracted from cell samples using well known, commercially available methods and reagents, and then subjected to reverse transcription and polymerase chain reaction using MAGE-1, MAGE-2, MAGE-3 and MAGE-4 specific primers. See Brasseur, et al, supra. Table 1, which follows, presents the results of this work. It shows that, regardless of status of MAGE-2, 3 or 4 expression both mAbs stained all MAGE-1 positive cells.

TABLE I - IMMUNOCYTOCHEMICAL REACTIVITY OF MAbs MCI AND 6F12 WITH VARIOUS CELL LINES

Cell lines	Immunostaining ¹		MAGE-mRNA expression ²
	MAb MCI	MAb 6F12	
(a) MZ2-MEL 3.1	+	+	1+, 2+, 3+, 4+
(b) MZ2-MEL 2.2	-	-	1-, 2+, 3+, 4-
(c) MZ2-MEL 2.2 ET1	+	+	1+, 2+, 3+, 4-
(d) Me235	+	+	1+, 2+, 3+, 4-
(e) Mi13443	+	+	1+, 2+, 3+, 4+
(f) NAK-MEL	-	-	1-, 2-, 3-, 4-
(g) Me220	-	-	1-, 2-, 3-, 4-
(h) Me241-2	+	+	1+, 2-, 3-, 4-
(i) M19	-	-	1-, 2+, 3+, 4-
(j) M113	-	-	1-, 2+, 3+, 4-
(k) M121	-	-	1-, 2-, 3+, 4-
(l) U251	-	-	1-, 2-, 3-, 4-
(m) MCF7	-	-	1-, 2-, 3-, 4-
(n) FeK4	-	-	1-, 2-, 3-, 4-
(o) P815	-	-	1-, 2-, 3-, 4-
(p) P815/MAOE-1	+	+	1+, 2-, 3-, 4-
(q) HEL	+	+	1+, 2-, 3-, 4-
(r) TFI	-	-	1-, 2-, 3+, 4-

(a) melanoma; (b) glioblastoma; (c) breast carcinoma; (d) fibroblast;
 (e) mouse mastocytoma; (f) mouse mastocytoma transfected with
 MAGE-1 cDNA; (g) myeloid leukemia. ¹ Acetone-fixed cells were
 stained by a 3-layer biotin/avidin/peroxidase system. ² The cellular
 mRNA was reverse transcribed and the cDNA tested by PCR
 using primers specific for MAGE-1, -2, -3 or -4 sequences.

Example 4

A further set of experiments were then carried out, using the well known Western blotting technique. Five cell lines were tested, i.e., MZ2-MEL 3.1, MZ2-MEL 2.2, MZ2-MEL 2.2 ET1, NA8 MEL, and Mi13443. All of these lines are presented in Table 1, supra. Cells were cultured, and then lysed in a Nonidet P40 (NP-40) buffer (150 mM NaCl, 0.5% NP-40, 2 mM EDTA, 80 mM Tris-HCl, pH 7.5, 0.02% NaN₃, 100 ug/ml PMSF and 100 ug/ml aprotinin). Approximately 50 ug aliquots were then subjected to SDS-PAGE under reducing conditions, and the thus separated proteins were transferred to nitrocellulose paper. Undiluted hybridoma supernatants, and a standard, commercially available electrochemiluminescence detection system was used. Figure 1 shows these results. The were intriguing because two major bands were found by both mAbs when testing MZ2-MEL

3.1. These bands are at 46 and 72 kilodaltons. The known
MAGE-1 specific monoclonal antibody MA454 (Chen, et al.,
Proc. Natl. Acad. Sci. USA 91: 1004-1008 (1994); U.S.
Patent No. 5,541,104)) did not detect anything in MAGE-1
negative cell line MZ2-MEL 2.2, but when this cell line was
transfected with MAGE-1 cDNA (to become cell line MZ2-MEL
2.2 ET1), MA 454 mAb did bind to a 46 kD band. one
concludes from this that the 46 kilodalton species bound by
all of MA454, 6C1, and 6F12, is MAGE-1 protein, but that
the latter two mAbs are cross reactive with a second, 72
kilodaltons protein which was expressed by MZ2-MEL 3.1,
MZ2-MEL 2.2, and Mi13443 (as well as transfected MZ2 MEL
2.2. ET1). Note, however, that MZ2-MEL 2.2 is MAGE-1
negative, suggesting that the cross reactivity is with a
non-MAGE-1 protein.

Example 5

The fact that NA8-MEL did not express any of MAGE-1,
2, 3 or 4 and did not produce any proteins which bound to
any of the three mAbs tested, made it useful in experiments
to determine whether or not detection of the 72 kDa protein
was dependent on presence of MAGE-1. The NA8-MEL cells
were transiently transfected with MAGE-1 cDNA in plasmid
pcDNAI, using lipofectin. The transfected cells were
lysed, and analyzed via Western blotting, as described
supra, using 6C1 and 6F12. A band of 46 kilodaltons
resulted, as did a faint band corresponding to what is
believed to be a multimeric form of MAGE-1. See figure 2.
No 72 kDa band was found, however. There was no 72 kDa
protein found following transient transfection with each of
MAGE-2, 3, 4 and 12. This was also true with COS-7 cells,
following transient transfection.

Example 6

In view of the unexpected presence of the 72 kDalton
band, Western blotting was carried out in accordance with
the procedures set forth supra, on a large number of cells.

The results are shown in Table 2, which also presents results from MAGE-1, 2, 3 and 4 mRNA expression testing. There was no relationship observed between the 46 and 72 kDalton proteins.

TABLE II - DETECTION OF MAGE-1 PROTEIN AND THE 72-kDa PROTEIN IN CELL LINES BY WESTERN BLOTTING WITH MAbs 6C1 AND 6F12

5

Cell lines	Western blot		MAGE-mRNA expression
	MAGE-1 protein	72-kDa protein	
(a)MZ2-MEL 3.1	+	+	1 ^o , 2 ^o , 3 ^o , 4 ^o
(a)MZ2-MEL 2.2	-	+	1-, 2 ^o , 3 ^o , 4-
(a)MZ2-MEL 2.2	+	+	1 ^o , 2 ^o , 3 ^o , 4-
ETI			
(a)Mi13443	+	+	1 ^o , 2 ^o , 3 ^o , 4 ^o
(a)NA8-MEL	-	-	1-, 2-, 3-, 4-
(a)IGR39	-	-	1-, 2-, 3-, 4-
(a)Me272.LN2	-	-	1-, 2-, 3 ^o , 4-
(a)Me220	-	-	1-, 2-, 3-, 4-
(a)IGR3	+	+	1 ^o , 2 ^o , 3 ^o , 4 ^o
(a)Mc204.A.1	-	-	1-, 2 ^o , 3 ^o , 4-
(a)Me242.B.1	-	-	1-, 2 ^o , 3-, 4-
(a)Me241.1	+	+	1 ^o , 2-, 3 ^o , 4-
(a)Me235	+	+	1 ^o , 2 ^o , 3 ^o , 4-
(a)Me192.2.2U	+	+	1 ^o , 2 ^o , 3 ^o , 4 ^o
(a)Mi9	-	-	1-, 2 ^o , 3 ^o , 4-
(a)Mi13	-	-	1-, 2 ^o , 3 ^o , 4-
(a)Mi21	-	-	1-, 2-, 3 ^o , 4-
(a)Me24H.3	+	+	1 ^o , 2 ^o , 3 ^o , 4 ^o
(a)Me244.1	+	+	1 ^o , 2 ^o , 3-, 4-
(a)Mc222.6	+	+	1 ^o , 2 ^o , 3 ^o , 4-
(a)M14	+	+	1 ^o , 2 ^o , 3 ^o , 4 ^o
(a)C1-18	+	-	1 ^o , 2-, 3 ^o , 4-
(a)LU251	-	-	1-, 2-, 3-, 4-
(a)LN215	+	+	1 ^o , 2 ^o , 3 ^o , 4-
(a)TF1	-	-	1-, 2-, 3 ^o , 4-
(a)HEL	+	-	1 ^o , 2-, 3-, 4-
(a)MCF7	-	-	1-, 2-, 3-, 4-
(a)ACN	+	-	1 ^o , 2 ^o , 3 ^o , 4-
(a)LAN-2	+	+	1 ^o , 2 ^o , 3 ^o , 4 ^o
(a)Fek4	-	-	1-, 2-, 3-, 4-

(^o)Melanoma; (^o)glioblastoma; (^o)myeloid leukemia; (^o)breast carcinoma; (^o)neuroblastoma; (^o)fibroblast.

Example 7

It is known that MAGE-1 expression can be induced, in vitro, in some MAGE-1 mRNA negative cell lines, by 5-aza-2'- deoxycytidine, a hypomethylating agent ("DAC"). This agent was incubated with three MAGE-1 mRNA negative cell lines (IGR 39, NA8-MEL, and U251), for 72 hours, after which lysates were taken, and incubated with monoclonal antibody 6C1. This treatment induced production of both the 46 kDa and the 72 kDa protein.

Example 8

The intriguing results reported supra suggested further experiments to determine the identify of the 72 kDa protein. First, a melanoma expression library was prepared from melanoma cell line MZ2-MEL 43, using a commercially available system. Following the preparation, bacteriophages were plated (approximately 4×10^5 pfus), and transferred to nitrocellulose filters. These were then blocked with 5% milk powder in phosphate buffered saline, and then incubated with monoclonal antibody 6C1 (hybridoma supernatant diluted 1:4 in RPMI/10% fetal calf serum). The materials were then washed with PBS/0.5% Tween-20 and incubated with horseradish peroxidase conjugated sheep anti-mouse IgG, diluted 1:3000 in PBS/5% milk powder in PBS. Another wash, with 5% Tween-20 followed. Signals were detected using ECL, as discussed supra. All positive plaques were subjected to secondary and tertiary screening. Positives were then picked and transferred to a tube containing phage lysis buffer (20 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.1% Tween 20), and an aliquot of this (5 ul) was used to amplify phage inserts. These were amplified via PCR, using:

GTGGCGACGA CTCCTGGAG (SEQ ID NO: 1)

and

CAGACCAACT GGTAAATGGTA GCG (SEQ ID NO: 2)

which are λ primers. The cycling parameters were:

1 minute at 94°C, 1 minute at 61°C, and 1 minute at 72°C, for 30 cycles, followed by a final extension at 72°C, for 10 minutes.

5 A partial 5' sequence of the clones was then obtained, using a commercially available sequencing kit, using SEQ ID NO: 1. See Casanova, Meth. Mol. Biol. 23: 191-197 (1993). Twelve clones were sequenced, and three were found to be identical to that of the MAGE-10 genomic sequence, as reported by DePlaen et al., Immunogenetics 40: 360-369
10 (1994), and Genbank Accession No. U10685.

One insert was then amplified, using SEQ ID NOS: 1 and 2, and a commercially available system. The cycling parameters for this amplification were 15 seconds at 94°C, 30 seconds at 61°C, 1 minute at 72°C (10 cycles), 15
15 seconds at 94°C, 30 seconds at 61°C, 80 seconds plus 20 seconds cycle elongation at 72°C, for 20 cycles followed by 10 minutes at 72°C, for a final extension. The amplification product was cleaved with restriction endonucleases NotI and SalI, and then subcloned into Bluescript plasmid. Automated sequencing was then carried
20 out using T3 and T7 primers. It was confirmed to be a partial MAGE-10 cDNA sequence (1400 base pairs), which corresponded to a start at position 2770 at the 5'-end, and extending 660 base pairs beyond the 3'-end of the genomic
25 sequence reported by DePlaen, et al., *supra*.

Example 9

Using the information obtained from the experiments described, *supra*, additional work was carried out to obtain a full length cDNA clone for MAGE-10.

30 As indicated, the partial cDNA clone was 1.4 kb long. This fragment was subjected to digestion with restriction endonucleases, and an HpaI fragment, corresponding to nucleotides 2770-3510 of the known, gDNA sequence, was isolated, and ³²P labeled, using a random priming DNA labeling kit. The labeled probe was then used to screen
35 two libraries from a melanoma cell line (Lyse-4), in the

vectors pcDNAI/Amp and pCEP4. The hybridization was carried out on filters, using 5xSSC, 5xDenhardt's, 0.5% SDS, and 100 ug/ml denatured salmon sperm DNA, at 65°C. Filters were then washed three times for 10 minutes at room 5 temperature, with 1xSSC, 0.1% SDS, once for 20 minutes at 65°C, with 1xSSC, 0.1% SDS, and twice for 20 minutes at 65°C, with 0.1xSSC, 0.1% SDS. Ten positive clones were found, and sequenced automatically, using T7 and SP6 primers for the pcDNA I/Amp vector, and the pCEP-4 forward 10 primer for pCEP-4. Several MAGE-10 clones were isolated, and fell into two categories (2.5 kb, and 1.5 kb, respectively), with different 3'-ends. The difference may result from alternate oligo (dT) priming during the cDNA 15 synthesis for the library. The clones all seemed to be identical but for the first 50-70 nucleotides at the 5'-end. Comparison to the known, genomic sequence delineated existence of at least four exons, the last two being identical to those predicted by DePlaen, et al. supra 20 (positions 1740-1814, and 1890-end). The second exon corresponded to positions 603-701, while the first exon did not appear to correspond to any previously recognized MAGE- 25 10 sequence. The open reading frame was found in the last exon. A sequence is set forth at SEQ ID NO: 3. The first 100 bases or so indicate consensus sequences, based upon the collective sequence information secured via these experiments.

Example 10

Three clones were isolated from the pcDNAI/Amp library, described supra, and were used for in vitro 30 transcription and translation. These inserts were about 1.5 kilobases long, terminating at about position 3156, using genomic sequence enumeration. One ug of each DNA was translated, using a commercially available system, and a luciferase control plasmid was used as control. 35 Translation products were subjected to PAGE analysis, and duplicate gels of non-radioactively labeled product were

transferred to membranes, where Western Blotting was carried out, using mAb 6C1, or polyclonal antibodies prepared as described infra. Radiolabelled materials showed a 72 kilodalton protein from all three clones tested, suggesting that the mAb was cross reactive with MAGE-1 and MAGE-10.

Example 11

Polyclonal antiserum against MAGE-10 was made as follows.

10 Immunogenic, MAGE-10 derived peptide

(H) QDRIATTDDTTAMASASSSATGSFSYPE (OH)

(SEQ ID NO: 4),

a portion of the deduced amino acid sequence of MAGE-10 was made, as were hybrids of this peptide and helper peptide P-
15 30.

Helper Peptide P30 is well known, as per Valmori, et al., J. Immunol. 149: 717-721 (1992). It is a tetanus toxin T cell epitope, with amino acid sequence:

FNNFTVSFWLRVPKVSASHLE

20 (SEQ ID NO: 5). Peptides were dissolved at 400 ug/ml in 100 mM Tris-HCl, pH 7.5, 0.9% NaCl. A rabbit was immunized over a 56 day period, with hybrid peptide (0.5 ml) at day 0, the MAGE-10 peptide (0.5 ml) at day 14, a second 0.5 ml injection of hybrid at day 28, and a final injection at day
25 56, of 0.5 ml of the MAGE-10 peptide.

Antiserum produced in accordance with this protocol was tested for reactivity with MAGE-10 in various assays. Specifically, the in vitro translation product of expression of cDNA corresponding to SEQ ID NO: 3 was tested in Western blotting experiments, along the lines set forth
30 supra. The antiserum was found to bind to a protein which was produced via the in vitro expression. It also recognized a 72 kDa band from melanoma lysates. In an ELISA, the polyclonal antibodies were found to recognize
35 the MAGE-10 peptide.

The foregoing experiments describe the production of monoclonal antibodies which specifically bind to a tumor rejection antigen precursor TRAP.

5 The invention thus relates to MAGE-10 binding monoclonal antibodies and the hybridomas which produce them. The mAbs were found to be useful in determining expression of MAGE-10. The mAbs can be added, e.g., in labeled form, bound to a solid phase, or otherwise treated to increase the sensitivity of MAGE-10 detection. Any of
10 the standard types of immunoassays, including ELISAs, RIAs, competitive assays, agglutination assays, and all others are encompassed with respect to the way the mAbs can be used. The detection of MAGE-10 expression product is useful, e.g., in diagnosing or monitoring the presence or
15 progression of a cancer.

20 The isolated, MAGE-10 protein is also a feature of this invention. This molecule has a molecular weight of about 72 kDa as determined by SDS-PAGE, and is useful as an immunogen as is the peptide of SEQ ID NO: 4, shown by the examples to be immunogenic. Preferably, these are used in combination with a suitable adjuvant.

25 Isolated cDNA encoding MAGE-10 is also a feature of this invention, such as the cDNA of SEQ ID NO: 3. Also a part of the invention are cDNA molecules which have complementary sequences that hybridizes to SEQ ID NO: 3 under stringent conditions (e.g., 0.2xSSC, 0.1% SDS at 65°C or, more preferably, 0.1xSSC). These should include, as a minimum, nucleotides 164-574 of SEQ ID NO: 3, in 5' to 3' order. Nucleic acid molecules consisting of nucleotides
30 164-185, and 553-574 of SEQ ID NO: 3 are especially useful as probes and/or primers, and are also a part of this invention. The sequences can be used in the form of expression vectors when operably linked to promoters, and then used to transform or transfect cells, to produce various recombinant eukaryotic cell lines and prokaryotic cell strains. Similarly, the sequences, and sequences such as SEQ ID NOS: 1 and 2 can be used in various hybridization
35

assays, such as PCR based assays. These are well known to the skilled artisan, and need not be repeated here.

The terms and expression which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expression of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

(1) GENERAL INFORMATION:

- (i) APPLICANTS: Rimoldi, Donata; Jongeneel, Victor; Coulie, Pierre; Cerrottini, Jean-Charles; Carrel, Stefan; Reed, Daryl
- (ii) TITLE OF INVENTION: MAGE-10 ENCODING cDNA, The Tumor Rejection Antigen Precursors Mage-10, Antibodies Specific To The Molecule, and Uses Thereof
- (iii) NUMBER OF SEQUENCES: 3
- (iv) CORRESPONDENCE ADDRESS:
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 - (C) CITY: New York City
 - (D) STATE: New York
 - (E) USA
 - (F) ZIP: 10022
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette, 3.5 inch, 144 kb storage
 - (B) COMPUTER: IBM
 - (C) OPERATING SYSTEM: PC-DOS
 - (D) SOFTWARE: Wordperfect
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/724,774
 - (B) FILING DATE: 03-October-1996
 - (C) CLASSIFICATION: 435
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Hanson, Norman D.
 - (B) REGISTRATION NUMBER: 30,946
 - (C) REFERENCE/DOCKET NUMBER: LUD 5457-PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (212) 688-9200
 - (B) TELEFAX: (212) 838-3884

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTGGCGACGA CTCCTGGAG

19

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CAGACCAACT GGTAATGATA GCG

23

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2559 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TCCGGGGTCG	CTCGAGCCGG	CCGGGACTCG	GGGATCASAA	GTAACGGCGG	50
YYMKYGTKCT	GAGGGACAGG	CTTGAGATCG	GCTGAAGAGA	GCGGGCCAG	100
GCTCTGTGAG	GAGGCAAGGG	AGGTGAGAAC	CTTGCTCTCA	GAGGGTGACT	150
CAAGTCAACA	CAGGGAACCC	CTCTTTCTA	CAGACACAGT	GGGTCGCAGG	200
ATCTGACAAG	AGTCCAGGTT	CTCAGGGAC	AGGGAGAGCA	AGAGGTCAAG	250
AGCTGTGGA	CACCACAGAG	CAGCACTGAA	GGAGAAAGACC	TGCCTGTGGG	300
TCCCCATCGC	CCAAGTCCTG	CCCACACTCC	CACCTGCTAC	CCTGATCAGA	350
GTCATCATGC	CTCGAGCTCC	AAAGCGTCAG	CGCTGCATGC	CTGAAGAAGA	400
TCTTCAATCC	CAAAGTGAGA	CACAGGGCCT	CGAGGGTGCA	CAGGCTCCCC	450
TGGCTGTGGA	GGAGGATGCT	TCATCATCCA	CTTCCACCAAG	CTCCTCTTTT	500
CCATCCTCTT	TTCCCTCCTC	CTCCTCTTCC	TCCTCCTCCT	CCTGCTATCC	550
TCTAATACCA	AGCACCCAG	AGGAGGTTTC	TGCTGATGAT	GAGACACCAA	600
ATCCTCCCCA	GAGTGCTCAG	ATAGCCTGCT	CCTCCCCCTC	GGTCGTTGCT	650
TCCCTTCCAT	TAGATCAATC	TGATGAGGGC	TCCAGCAGCC	AAAAGGAGGA	700
GAGTCCAAGC	ACCCTACAGG	TCCTGCCAGA	CAGTGAGTCT	TTACCCAGAA	750
GTGAGATAGA	TGAAAAGGTG	ACTGATTGG	TGCAGTTCT	GCTCTTCAAG	800
TATCAAATGA	AGGAGCCGAT	CACAAAGGCA	GAAATACTGG	AGAGTGTCA	850

AAAAAAATTAT	GAAGACCAC	TCCCTTGTT	GTTTAGTGAA	GCCTCCGAGT	900
GCATGCTGCT	GGCTTTGGC	ATTGATGTAA	AGGAAGTGGA	TCCCAC TG GC	950
CACTCCTTG	TCCTTGTCAC	CTCCCTGGC	CTCACCTATG	ATGGGATGCT	1000
GAGTGATGTC	CAGAGCATGC	CCAAGACTGG	CATTCTCATA	CTTATCCTAA	1050
GCATAATCTT	CATAGAGGGC	TACTGCACCC	CTGAGGAGGT	CATCTGGGAA	1100
GCACTGAATA	TGATGGGGCT	GTATGATGGG	ATGGAGCACC	TCATTTATGG	1150
GGAGGCCAGG	AAGCTGCTCA	CCCAAGATTG	GGTGCAGGAA	AACTACCTGG	1200
AGTACCGGCA	GGTGCCTGGC	AGT GAT C CTG	CACGGTATGA	GTTCTGTGG	1250
GGTCCAAGGG	CTCATGCTGA	AATTAGGAAG	ATGAGTCTCC	TGAAATTTT	1300
GGCCAAGGTA	AATGGGAGTG	ATCCAAGATC	CTTCCC ACTG	TGGTATGAGG	1350
AGGCTTGAA	AGATGAGGAA	GAGAGAGCCC	AGGACAGAAT	TGCCACCACA	1400
GATGATACTA	CTGCCATGGC	CAGTCAAGT	TCTAGCGCTA	CAGGTAGCTT	1450
CTCCTACCCCT	GAATAAGTA	AGACAGATTG	TTCACTGTGT	TTTAAAAGGC	1500
AAGTCAAATA	CCACATGATT	TTACTCATAT	GTGGAATCTA	AAAAAAAAAA	1550
AAAAAAAAGT	TGGTATCATG	GAAGTAGAGA	GTAGAGCAGT	AGTTACATTA	1600
CAATTAAATA	GGAGGAATAA	GTTCTAGTGT	TCTATTGCAC	AGTAGGATGA	1650
CTATAGTTAA	CATTAAGATA	TTGTATATTA	CAAAACAGCT	AGAAGGAAGG	1700
CTTTTCAATA	TTGTCACCAA	AAAGAAATGA	TAAATGCATG	AGGTGATGGA	1750
TACACTACCT	GATGTGATCA	TTATACTACA	TATA CATGAA	TCAGAACATC	1800
AAATTGTACC	TCATAAAATAT	CTACAATTAC	ATGTCAGTTT	TTGTTTATGT	1850
TTTTGTTTT	TTTAATTAA	TGAAAACAAA	TGAGAATGGA	AATCAATGAT	1900
GTATGTGGTG	GAGGGCCAGG	CTGAGGCTGA	GGAAAATACA	GTGCATAACA	1950
TCTTTGTCTT	ACTGTTTCT	TTGGATAACC	TGGGGACTTC	TTTTCTTTTC	2000
TTCTTGGTAT	TTTATTCTCT	TTTCTTCTT	CTTCTTTTT	TTTTTTAACAA	2050
AAGTCTCACT	CTATTGCTCT	GGCAGGGAGTG	CAGTGGTGCA	GTCTCGGCTC	2100
ACTGCAACTT	CCGCCTCCTG	GGTTCAAGCG	ATTCTCCTGC	CTCAGTCTCC	2150
TGAGTAGCTG	GGATTACAAG	TGTGCACCA	CATA CCCGGC	TAATTTGTAA	2200
TTTTTTAGTA	GAGATGGGGT	TTCACCATGT	TGGCCAGGCT	GGTCTCAAAC	2250
TCCTGACCTC	AGGTAATCTG	CCCGCCTCAG	CCTCCCAAAG	TGCTGGGATA	2300
ACAGGGTGTGA	GCCCAC TGCA	CCCCAGCCTC	TTCTTGGTAT	TTAAAATGT	2350
TGTTACTTTT	ACTAGAATGT	TTATGAGCTT	CAGAATCTAA	GGTCACACGT	2400
TCGTTCTGT	TTATCCAGTT	TAAGAAACAG	TTTGCTATT	TTGTAAAACA	2450
AATTGGGAAC	CCTTCCATCA	TATTGTAAT	CTTTAATAAA	ATAACATGGA	2500
ATTGGAATAG	TAATTTCTT	GGAAATATGA	AAAAATAGTA	AAATAGAGAA	2550
AATAATT	TT				2559

We claim:

1. Isolated cDNA molecule which encodes MAGE-10 tumor rejection antigen precursor, the complementary sequence of which hybridizes to nucleotides 164-574 SEQ ID NO: 3, under stringent conditions.
2. The isolated cDNA molecule of claim 1, comprising nucleotides 67-2559 of SEQ ID NO: 3.
3. The isolated cDNA molecule of claim 1, comprising SEQ ID NO: 3.
4. Expression vector comprising the isolated cDNA molecule of claim 1, operably linked to a promoter.
5. Eukaryotic cell line or prokaryotic cell strain, transfected or transformed with the expression of claim 3.
6. Isolated MAGE-10 tumor rejection antigen precursor having a molecular weight of about 72 kilodaltons as determined by SDS-PAGE.
7. Isolated MAGE-10 tumor rejection antigen precursor encoded by the isolated cDNA molecule of claim 1.
8. Monoclonal antibody which binds to the isolated tumor rejection antigen precursor of claim 6.
9. Isolated tumor rejection antigen precursor derived peptide consisting of the amino acid sequence of SEQ ID NO: 4.
10. Immunogenic composition comprising the isolated MAGE-10 tumor rejection antigen precursor of claim 6, and an adjuvant.

11. Immunogenic composition comprising the peptide of claim 9 and an adjuvant.

12. Polyclonal antiserum obtained by immunizing a non-human animal with the peptide of claim 8 under conditions favoring an immune response thereto, and isolating antiserum produced thereby.

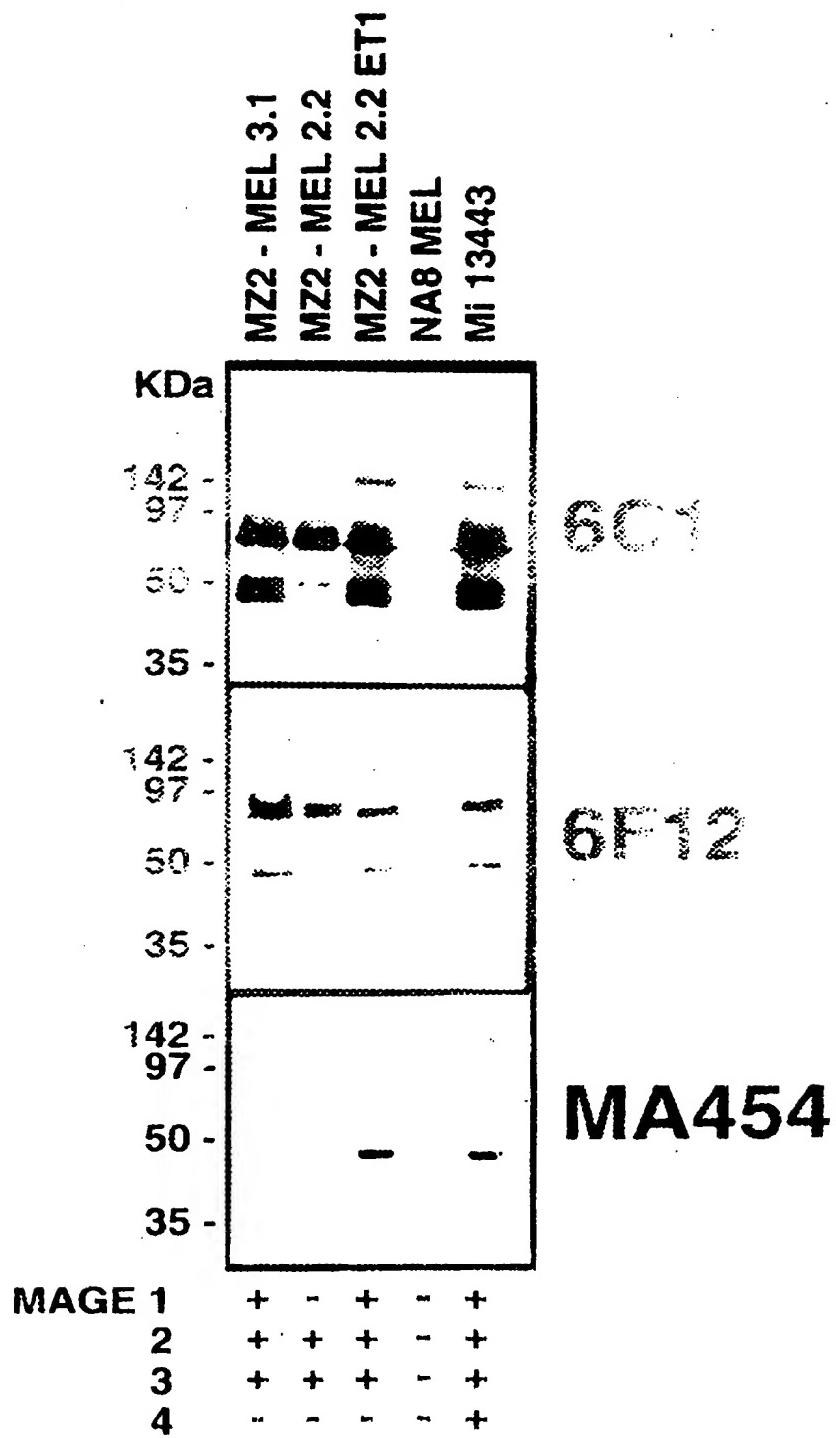
13. Method for determining presence of MAGE-10 tumor rejection antigen precursor in a sample, comprising contacting said sample with the monoclonal antibody of claim 8 and determining binding therebetween.

14. Method for determining presence of MAGE-10 tumor rejection antigen precursor in a sample comprising contacting said sample with the polyclonal antiserum of claim 12, and determining binding therebetween.

15. Hybridoma cell in which produces the monoclonal antibody of claim 8.

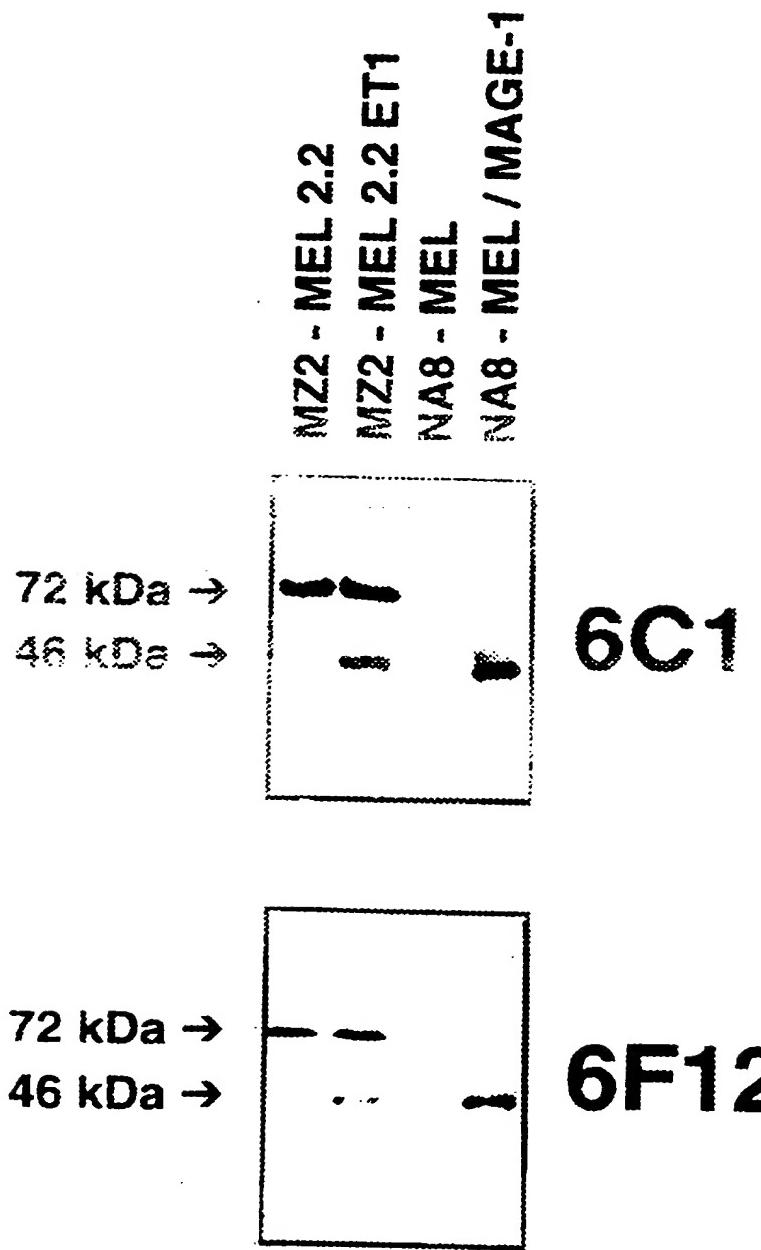
16. The monoclonal antibody of claim 8, wherein said antibody is humanized.

1/2

FIG. 1

SUBSTITUTE SHEET (RULE 26)

2/2

FIG. 2

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/15981

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04; C12N 1/20; G01N 33/53; C07K 16/00
US CL : 536/23.1; 435/252.3, 7.1; 530/387.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 435/252.3, 7.1; 530/387.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MPSRCH

Search terms: MAGE-10, tumor rejection antigen precursor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE PLAEN et al. Structure, chromosomal localization, and expression of 12 genes of the <i>MAGE</i> family. Immunogenetics. 1994, Vol. 40, pages 360-369, see entire document.	1, 4-5
X	WO 95/23874 A1 (LUGWIG INSTITUTE FOR CANCER RESEARCH) 08 September 1995, see entire document.	1, 4-5
X -- Y	WO 92/20356 A1 (LUDWIG INSTITUTE FOR CANCER RESEARCH) 26 November 1992, see entire document, and especially claims 109-110.	1, 4-5, 8, 12, 15 ----- 13-14, 16

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

17 DECEMBER 1997

Date of mailing of the international search report

10 FEB 1998

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/15981

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please see Extra Sheet.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.



No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/15981

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING (Continued)

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-7, 9-11, 13, 14, drawn to an isolated cDNA molecule encoding MAGE-10 tumor rejection antigen precursor, an expression vector comprising said cDNA molecule, a host cell transformed with said vector, an isolated MAGE-10 tumor rejection antigen precursor, an isolated peptide derived from said antigen precursor, and a method of detection of said MAGE-10 tumor rejection antigen precursor.

Group II, claim(s) 8, 12, 15, 16, drawn to a monoclonal, or polyclonal, or humanized antibody which binds to said MAGE-10 tumor rejection antigen precursor, and a hybridoma cell line producing said monoclonal antibody.

and it considers that the International Application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

An international stage application shall relate to one invention only or to a group of invention so linked as to form a single general inventive concept. If multiple products, processes of manufacture or uses are claimed, the first invention of the category first mentioned in the claims of the application will be considered as the main invention in the claims, see PCT article 17(3) (a) and 1.476 (c), 37 C.F.R. 1.475(d). Group I will be the main invention. After that, all other products and methods will be broken out as separate groups (see 37 CFR 1.475 (d)).

Group I, claims 1-7, 9-11, 13-14 form a single inventive concept. Group II is an additional product, i.e., an antibody against MAGE-10 tumor rejection antigen precursor.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07H 21/04, C12N 1/20, G01N 33/53, C07K 16/00		A1	(11) International Publication Number: WO 98/14463 (43) International Publication Date: 9 April 1998 (09.04.98)
(21) International Application Number: PCT/US97/15981 (22) International Filing Date: 10 September 1997 (10.09.97)		(81) Designated States: AU, CA, CN, JP, KR, NZ, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 08/724,774 3 October 1996 (03.10.96) US		Published <i>With international search report.</i> <i>With amended claims.</i>	
(71) Applicant: LUDWIG INSTITUTE FOR CANCER RESEARCH [CH/US]; 1345 Avenue of the Americas, New York, NY 10105 (US).		Date of publication of the amended claims: 4 June 1998 (04.06.98)	
(72) Inventors: RIMOLDI, Donata; 155, chemin des Boveressis, CH-1066 Epalinges (CH). JONGENEEL, Victor; 155, chemin des Boveressis, CH-1066 Epalinges (CH). COULIE, Pierre; 74, avenue Hippocrate, UCL 7459, B-1200 Brussels (BE). CERROTTINI, Jean-Charles; 155, chemin des Boveressis, CH-1066 Epalinges (CH). CARREL, Stefan; 155, chemin des Boveressis, CH-1066 Epalinges (CH). REED, Daryl; 155, chemin des Boveressis, CH-1066 Epalinges (CH).			
(74) Agent: HANSON, Norman, D.; Felfe & Lynch, 805 Third Avenue, New York, NY 10022 (US).			
(54) Title: MAGE-10 ENCODING cDNA, THE TUMOR REJECTION ANTIGEN PRECURSOR MAGE-10, ANTIBODIES SPECIFIC TO THE MOLECULE, AND USES THEREOF			
(57) Abstract <p>Isolated cDNA molecules which encode the tumor rejection antigen precursor MAGE-10, the protein itself, antibodies to it, and uses of these are a part of the invention.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

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DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

AMENDED CLAIMS

[received by the International Bureau on 9 April 1998 (09.04.98);
original claims 1-5 amended; new claims 17 and 18 added; remaining claims unchanged (2 pages)]

1. Isolated cDNA molecule which encodes MAGE-10 tumor rejection antigen precursor having a molecular weight of about 72 kilodaltons as determined by SDS-PAGE, the complementary sequence of which hybridizes under stringent conditions to nucleotides 164-574 of SEQ ID NO: 3.
2. The isolated cDNA molecule of claim 1, comprising nucleotides 67-2559 of SEQ ID NO: 3.
3. The isolated cDNA molecule of claim 1, comprising SEQ ID NO: 3.
4. Expression vector comprising the isolated cDNA molecule of claim 1, operably linked to a promoter.
5. Eukaryotic cell line or prokaryotic cell strain, transfected or transformed with the expression vector of claim 3.
6. Isolated MAGE-10 tumor rejection antigen precursor having a molecular weight of about 72 kilodaltons as determined by SDS-PAGE.
7. Isolated MAGE-10 tumor rejection antigen precursor encoded by the isolated cDNA molecule of claim 1.
8. Monoclonal antibody which binds to the isolated tumor rejection antigen precursor of claim 6.
9. Isolated tumor rejection antigen precursor derived peptide consisting of the amino acid sequence of SEQ ID NO: 4.
10. Immunogenic composition comprising the isolated MAGE-10 tumor rejection antigen precursor of claim 6, and an adjuvant.

11. Immunogenic composition comprising the peptide of claim 9 and an adjuvant.

12. Polyclonal antiserum obtained by immunizing a non-human animal with the peptide of claim 8 under conditions favoring an immune response thereto, and isolating antiserum produced thereby.

13. Method for determining presence of MAGE-10 tumor rejection antigen precursor in a sample, comprising contacting said sample with the monoclonal antibody of claim 8 and determining binding therebetween.

14. Method for determining presence of MAGE-10 tumor rejection antigen precursor in a sample comprising contacting said sample with the polyclonal antiserum of claim 12, and determining binding therebetween.

15. Hybridoma cell in which produces the monoclonal antibody of claim 8.

16. The monoclonal antibody of claim 8, wherein said antibody is humanized.

17. Eukaryotic cell line transfected with the expression vector of claim 3.

18. Prokaryotic cell strain transformed with the expression vector of claim 3.